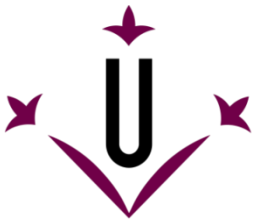

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Labile trace metal concentration measurements in marine environments: from coastal to open ocean areas

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ABSTRACT

In seawater, trace metals occur in many forms: free ion, labile and non-labile complex, colloid, associated with particles, but only free ions and very labile metal complexes can cross the phytoplankton membrane and be assimilated. In this paper we review the most appropriate determination methods for those metal species, addressing particularly in situ speciation and preconcentration. Focus is on DGT-ICPMS, which is a technique that meets these criteria very well. In coastal environments, DGT probes are widely used for metal pollution monitoring, but in open ocean a carrier such as a glider, is required. In open ocean organic metal complexation has been studied using DGT-ICPMS and electrochemical methods. The use of DGTs with different diffusion domain thicknesses provides information to quantify labilities and dissociation rates of metal complex pools. Labilities of metal complexes in coastal waters appeared to be higher than in open sea.

Keywords: metal complexes; lability; DGT; coastal areas; open ocean

1. INTRODUCTION

Coastal marine ecosystems can suffer from high metal inputs which are generally related to a variety of anthropogenic activities. However, the toxicity of each trace metal not only depends on its concentration, but also on the chemical form under which it is present in the seawater. Tetraethyl lead and methylmercury have no metabolic function and are toxic to organisms. Copper, cobalt, iron or zinc are micro-nutrients at low levels, but become toxic when their concentrations are high. For example, the toxicity effect to phytoplankton (unicellular organisms) has been correlated with the concentrations of free aquated metal ion and of the very labile metal complexes [1]. The latter are often complexes with inorganic ligands which dissociate in the diffusive boundary layer around the plankton cell [2].

In open ocean, trace metals such as Fe, Co or Mn have a totally different function on the ecosystem. They are present at very low concentrations, much lower than in coastal waters, and are limiting phytoplankton growth in the so-called High Nutrient Low Chlorophyll (HNLC) oceanic areas. Moreover, a determination of the concentrations of the labile trace metals in the marine environment is a real challenge, because the labile fraction is sometimes just a small percentage of the total dissolved burden. For example, labile Co, Mn and Fe concentrations in the Southern Ocean are at the pmol L^{-1} level [3]. It has been shown that the bioavailable fraction generally corresponds to free metal ions and the most labile trace metal complexes [2].

Analytical methods that allow the determination of labile trace metal complexes at low concentrations (nmol L^{-1} or even pmol L^{-1}) are very limited: (1) anodic stripping

voltammetry (ASV) and (2) diffusive gradient in thin films (DGT) with inductively coupled plasma mass spectrometry (ICP-MS). A technique that is more and more applied for the determination of dissolved Fe is flow injection with chemiluminescence detection (FIA-CL) [4]. It has also been sporadically applied for the determination of other metals, but it is not clear whether FIA-CL provides the total dissolved metal burden or a non-defined fraction of it. Each of the three techniques presents advantages and disadvantages, though only DGT-ICPMS allows the simultaneous measurement of all labile trace metals. With ASV, Cu, Cd and Zn can be measured simultaneously [5], while with FIA-CL a single metal is determined. In addition, ASV and FIA-CL necessitate, prior to analysis, the collection of a certain water volume. Sampling and sample handling (especially when dealing with very low concentrations as those in the open ocean) can lead to contamination and perturbation effects on the distribution of labile and non-labile species. The *in situ* sampling of the labile trace metals with DGT avoids these artifacts. Therefore, we will focus on DGT-ICPMS, since it is the most advanced technique for trace metal dynamic speciation in the marine environment.

A variety of DGT samplers have been used, depending on the species that must be analyzed and the intended goals. Generally, agarose or polyacrylamide is used for the diffusive gel, while a series of binding can be employed, as the following list shows: Chelex-100 and SPR-IDA (e.g. Cd, Cu, Pb, Mn, Fe, Zn and Ni) [6], 8-hydroxyquinoline (e.g. Cd, Fe, Mn, Ni and U) [7], a group of thiol based resins (e.g. Hg, MeHg) [8], Eichrom or a manganese dioxide based resin (e.g. Ra and transition metals) [9, 10], Metsorb a titanium dioxide based resin (oxyanionic species As, Sb, Mo, V, W) [10], Ferrihydrite an iron oxide based resin (oxyanionic species As, Sb, P, Se, V) [8] and ZrO based (cations

and oxyanions) [8]. For trace-metal dynamic speciation in the marine environment, Chelex-100 is the most frequently used DGT resin. The DGT technique is thus particularly suited for trace metal speciation in aquatic systems, but its application in a coastal or an open ocean environment requires adapted supporting infrastructure. For example, buoys, moorings or tripods can be used in coastal areas and new tools such as gliders can be used in open oceans.

In the marine environment, one can consider free metal ions and various metal-complex pools (Fig. 1), each with a typical lability (i.e. with a certain tendency to dissociate when the complex approaches a sink of free metal). The lability degree quantifies the current contribution of the complexes compared to the maximum one, which is complete dissociation [11]. These lability degrees can be determined by using DGTs with various thicknesses of the diffusive domain. Only free metal and highly labile metal complexes will contribute to the accumulation when using a thin diffusive domain, while thicker diffusive domains will also allow the contribution of less labile metal complexes [12, 13]. In addition, modelling of reaction-diffusion-accumulation will not only provide lability degrees of the metal complexes, but also information about their dissociation kinetics. Use of hydrogels with different pore sizes, for example open pore size (about 10 nm) and restricted pore size (about 1 nm), can distinguish between metal complexes of different size ranges [14].

Figure 1.

In this review paper, the use of different types of DGTs (regarding the thickness of the diffusive domain or the hydrogel pore size) in the marine environment to determine

trace metal complexes with various lability degrees is discussed. Other techniques will only be mentioned when they give more information or a better insight on the speciation, In the coastal marine area, high concentrations of toxic elements are a concern for regulating bodies, so, the capabilities of DGT to predict the accumulation of trace metals by organisms is also reviewed. The speciation in the Belgian Coastal Zone (BCZ) is included as a case study. Labile trace metal concentrations are compared to total dissolved ones, because labile and non-labile fractions can vary strongly between metals. In open sea, the greatest challenge is to determine micronutrients for plankton growth in HNLC areas: the few studies about labile trace metals in open sea that exist will be reviewed. Finally, modelling of reaction- diffusion-accumulation gives us insight in the lability of the different metal complex pools.

2. PASSIVE SAMPLERS

2.1 General aspects

The DGT technique relies on controlled diffusive transport of the analyte and is either used in the water column [3, 15] or inserted vertically in sediments [16] to determine high-resolution profiles of trace element concentrations. It generally utilizes two hydrogel layers: a polyacrylamide hydrogel that is backed up by a second polyacrylamide hydrogel layer containing the metal-selective Chelex cation-exchange resin (Fig. 2). The polyacrylamide hydrogel, the filter membrane (pore 0.45 μm) covering it and the diffusive boundary layer (DBL), form the diffusive domain in which labile metal complexes can dissociate. The degree of dissociation of the metal complexes depends on the thickness of that diffusive domain. Hence, only the metal complexes, small enough to diffuse through

151 the gel and capable of dissociation in the diffusive domain will be assessed (Fig. 3). The
152 amount of trace element that is bound to the resin is eluted by concentrated nitric acid and,
153 then, quantified by atomic absorption spectrometry (AAS), X-ray Fluorescence (XRF) or
154 Inductively Coupled Plasma-Mass Spectrometer (ICPMS) depending on the concentration
155 level.

156 Figure 2.

157 The characteristics of all DGT types discussed in this paper are summarized in
158 Table 1.Diffusion of metal complexes with fulvic and humic acid indicated pore sizes for
159 Open-Pored DGT gels of ~10 nm and for Restricted-Pored DGT gels of ~1 nm [14].
160 Further in this paper, we will name the open-pored size DGT (~10 nm pore-size) with a
161 0.8 mm hydrogel diffusion layer, the classic DGT, while the intermediate DGT has a
162 hydrogel layer of 0.4 mm thickness and the fast or reversed DGT has no hydrogel layer.
163 Since the thickness of the diffusive domain in the fast and intermediate DGT is smaller
164 than in the classic one, these samplers collect a higher amount of metal in the same time
165 of exposure. The restricted DGT has the same diffusion domain as the classic one, but the
166 hydrogel has only a pore-size of ~1 nm.

167 Table 1

168 Figure 3.

169 2.2 Concentration calculation

Knowing the metal amount accumulated in the resin (M in equation 1), the metal DGT-concentrations in the sea water can, then, be calculated using Fick's first diffusion law in case of perfect sink conditions [15, 16].

$$c_{DGT} = M\,\delta_g/(D_M\,A\,t) \qquad (1)$$

where c_{DGT} is the DGT-labile trace metal concentration in the seawater, δ_g is the aggregated thickness of diffusion domain: the filter membrane (around 0.125 mm) + the thickness of the diffusive hydrogel layer (0.8 mm for the classic and restricted DGT, no hydrogel layer for the fast DGT) + the thickness of DBL, D_M is the diffusion coefficient of the trace metal in the diffusion domain (assumed to the same in the gels and in the DBL), A is the exposure area of the diffusive layer to the sea water and t is the deployment time. The diffusive boundary layer thickness (DBL) depends on the turbulence in the aquatic system in which the DGT sampler is immersed. The typical DBL thickness is between 0.2 to 0.5 mm according to Davison and Zhang [17].

However, perfect sink conditions are not always met, such as, for example, DGT accumulation of Mn in seawater or in other solutions with high Ca or Mg concentrations [18]. In that case, Mn concentration calculations should be carried out with a modified equation 1, taking into account the competition of Mn with Ca and Mg ions for the resin sites, or via specific calibration curves of Mn (same solution composition and same exposure time as for field samples) accumulated on the DGT resin versus the Mn concentration in seawater.

2.3 Use of different DGT types

192 2.3.1 Classic versus restricted DGT:

193 Pore size of the classic DGT diffusive gel is around 10 nm, while it is only 1 nm
194 for the restricted one [14]. This means that most large organic metal complexes such as
195 metals bound to humic ligands cannot cross or only partially pass through the small pores
196 of the restricted DGT [19]. Inorganic metal complexes and free metal ions are smaller, they
197 can thus pass through the small pores and are bound to the resin of the restricted DGT.
198 Generally, mineral colloids cannot enter the small pores, neither of the restricted or the
199 open-pore DGT, and if they do, they generally will not dissociate in the diffusive domain.
200 Diffusion coefficients in restricted pore size hydrogel are smaller than in open pore size
201 hydrogel [20, 21].

202 2.3.2 Classic versus fast DGT:

203 Under perfect sink conditions, free metal arriving at the resin domain is
204 instantaneously trapped by the resin. Fully labile metal complexes entirely dissociate at the
205 resin-diffusive gel interface where the free metal is trapped by the resin, but the outcome
206 of partially labile metal complexes is different: the dissociation rate constant of the metal
207 complex and the residence time in the diffusion domain determine the extent by which the
208 metal is trapped by the resin. The residence time in a diffusion domain of 1.125 mm (classic
209 DGT) and of 0.325 mm (fast DGT) can be estimated with the Einstein-Smoluchowski
210 expression:

211 $t = 0.5(\delta^g)^2/D$ equation (2)

212 where t is the residence time in the diffusion domain, δ^g is the total thickness of the
213 diffusive domain (DBL of 0.2 mm; membrane of 0.125 mm; hydrogel of 0.8 mm for the

214 classic DGT and 0 for the fast one) and *D* is the diffusion coefficient of the compound in
215 the diffusive domain.

216 Using, for example, a typical *D* of 2×10^{-6} cm^2 s^{-1} for a metal complex, the residence
217 time of the complex in the diffusion domain is of the order of 4 min for a fast DGT and of
218 the order of 53 min for the classic DGT. In Anodic Stripping Voltammetry (ASV), the
219 diffusion domains around the electrode are much smaller than for DGTs. The diffusion
220 layer thickness is around 20 μm for a macroelectrode and 4.5 μm for a microelectrode [22].
221 When using a vibrating electrode during the deposition step, the diffusion layer even
222 decreases to 1-2 μm [23]. For a diffusive layer of 20 μm, the residence time becomes 1 s,
223 and for the vibrating electrode it is of the order of a millisecond. These residence times are
224 by far smaller than for the DGT, hence metal complexes have very little time to dissociate
225 and almost only free ions or extreme labile inorganic complexes will be measured.

226 Although the above overview allows to justify the different behaviour of complexes
227 on DGT devices with different thickness of the gel layer, this is a very simplified model of
228 what happens. Actually, net dissociation only takes places in the reaction layer, a layer
229 where equilibrium of the complexation reaction is not fulfilled. Differing from the
230 behaviour in electrodes, the reaction layer in DGT devices extends to both sides of the
231 resin-diffusive gel interface [12]. Indeed, in the diffusive hydrogel, the reaction layer
232 usually only spans a small fraction of the gel thickness, but net dissociation and diffusion
233 of partially labile complexes also take place in the resin domain, where they penetrate up
234 to an effective depth depending on the thickness of the resin disc and on the dissociation
235 rate constant [13]. It has been shown that the main contribution of the accumulation comes

from dissociation in the resin domain [24]. Quantitatively, this contribution depends on the local complex concentration in the resin and on the thickness of the reaction layer which at the end depends on the thickness of the diffusive domain. This is an easily varied parameter that it is here used to gain information on the dynamic characteristics of the speciation present.

3. APPLICATION OF DGTs IN MARINE ENVIRONMENTS:

3.1 Coastal environments

In many countries, regulations exist to reduce the pollution level in coastal environments. To achieve this, pollution monitoring tools are needed and DGT samplers have been particularly tested for that purpose.

3.1.1 Comparison between DGT and bio-indicators:

Several studies focused on the use of a DGT to mimick the biological system. The accumulation of trace metals by DGT and specific organisms such as bivalves, macroalgae or macroinvertebrates was compared: (1) In Ulsan Bay (East Sea, Korea) [25], spatial metal gradients in the seawater were much better reflected by DGTs than by mussels. In fact, the accumulation by mussels was controlled by physiological factors and partly by the diet. Thus, the result obtained with the mussels is also related to particulate metals, while DGTs only accumulate dissolved labile trace metals, which includes the fraction with the highest toxicity; (2) In Southeastern Brazilian coastal waters, labile and bioavailable concentrations of Cd, Co, Cu, Mn, Ni and Pb in seawater from Ilha Grande Bay, RJ were assessed using DGT and transplanted bivalves [26]. Labile trace metal data obtained with

the DGT technique were in most cases correlated to the bioavailable fraction in transplanted bivalves; (3) Bioavailable metals in a fjord near a former Pb–Zn mine in West Greenland were assessed using transplanted seaweed, mussels and sea snails and passive DGT samplers [27]. After a 9-day deployment period, concentrations of especially Pb, Zn and Fe in the species were all markedly elevated at the monitoring sites closest to the mine. Lead concentrations in all three species and the DGT-Pb results showed a significant linear correlation. Zinc and Fe concentrations were less correlated indicating that the mechanisms for Zn and Fe accumulation in the three species are more complex; (4) In the coastal waters of Sardinia (Italy) a comparison was made between DGTs and macroalgae as indicators of available trace metals in seawater [28]. They found that Pb and Cd levels were high enough to be of environmental concern in the whole area. A statistically significant correlation (p<0.05) between DGT-labile metal levels and those in *P. pavonica* (a macroalgae) was found for Pb and Cd (with the remark that for Cd, the experimental points were not evenly distributed over the entire concentration range); (5) Amirbahman et al. [29] studied mercury bioavailability for macroinvertebrates in estuarine sediments (Penobscot River estuary in Winterport, ME, USA). Their results suggest that (a) sediment MeHg is more bioavailable than inorganic Hg, (b) sediment and pore-water concentration measurements are not good predictors for the extent of bioaccumulation of Hg species, and (c) DGTs are an effective monitoring tool for the assessment of bioavailability of Hg species [29].

3.1.2 Application of the DGT in coastal waters:

Current environmental monitoring programs require a relatively large volume of samples for further analysis in the laboratory. This conventional approach measures only

momentary characteristics of the environment. It also presents several problems, such as the risk of sample contamination, the difficulty of preserving samples, and changes of solute species during the period in which the samples are preserved. The examples given in section 3.2.1, prove that DGT probes (which provide Time Weighted Average concentrations) are particularly suited for metal pollution monitoring in coastal areas, although a straightforward relation with specific organisms is not always observed, mainly due to physiological factors [25, 27]. The potential of the DGT technique as a monitoring tool for labile trace metal concentrations in dynamic estuarine and coastal waters, which are complex environments, is also confirmed by several studies.

A study of the Gold Coast Broadwater, Australia, demonstrated that important information could be obtained from intensive sampling of physicochemical parameters and trace metal concentrations [30]. A regular pattern of variation in Cu and Ni concentrations was related to point sources, rather than due to conventional tidal estuarine mixing of end-member waters. However, this approach was logistically demanding and expensive. The DGT technique was used as an alternative method due to its continual time integrated response to changes in trace metal concentrations. Significant correlations were found between 24 h DGT-labile measurements and 0.45-μm filterable measurements, on time-averaged composite samples (grab samples combined every 4 h for 24 h), for Cu (n = 24, r = 0.965, p < 0.001), Pb (n = 24, r = 0.799, p < 0.001), Zn (n = 17, r = 0.909, p < 0.001), and Ni (n = 23, r = 0.916, p < 0.001). DGT-labile measurements as a fraction of 0.45 μm filterable concentrations were 21 ± 2% for Cu, 29 ± 11% for Pb, 28 ± 5% for Zn, and 27 ± 12% for Ni, demonstrating the speciation capabilities of DGT. Although DGT measurements were confirmed as being operationally defined regarding the lability of

measured species, DGT was still found to be very promising as a monitoring approach, particularly for dynamic estuarine waters.

Speciation of trace metals (Zn, Cd, Pb, Cu, Ni and Co) within a small marine harbor of the Adriatic Sea (Rijeka, Croatia) was carried out [31]. Concentrations of dissolved metals were relatively low and comparable to, or slightly above those found in coastal Adriatic region. Compared to higher variability of dissolved metal concentrations in discrete grab sampling, a smoother temporal distribution was recorded for DGT-labile metals. The percentages of DGT-labile to Total Dissolved (TD) metal concentrations varied among metals: Cu (32-54%), Zn (45-56%), Co (52-56%), Ni (72-86%), Cd (70-90%) and Pb (75-105%). DGT-labile metal concentrations predicted by speciation modeling (a NICA-Donnan model is applied for modelling of metal-organic matter interactions) were in a good agreement with the measured ones for Zn, Cd and Ni, while they are underestimated for Pb and Cu, and overestimated for Co [31].

Measurements of total, filterable and DGT-labile concentrations of nine metals (Al, Cd, Cr, Cu, Fe, Pb, Mn, Ni and Zn) have been made at five sites up to 4.2 km from a deep sea tailings outfall at Lihir Island, Papua New Guinea [32]. At each site, pairs of DGT units (one containing a 0.4 mm and the other a 0.8 mm diffusive gel layer) were deployed at three depths for 4 to 7 days. Comparison of results obtained with 0.4 and 0.8 mm thick diffusive gels at sites distant and close to the outfall (within ~1 km) indicated a relative increase in bulk water concentrations at sites close to the outfall for DGT samplers with 0.8 mm diffusive gels. The authors attributed the cause of this effect to a previously undescribed process involving diffusion of colloidal iron and aluminium oxyhydroxides

through the diffusive gel layer. Their adsorbed or occluded metals are released slowly (either through equilibration or ageing of the colloids) to be bound on the Chelex resin. There is a greater likelihood of this in the thicker gels. While kinetic effects involving slow dissociation of an unidentified metal–ligand complex cannot be discarded completely, it is considered a less likely explanation. The thicker gels have a longer residence time over which metal ions can be released. Total and filterable metal (FM) concentrations were frequently below the limits of detection (LOD) achievable by conventional ICP-AES (1 to 52 $\mu g\ L^{-1}$) and this limited their usefulness for assessing environmental risk and for metal speciation determination [32].

3.2 Open Ocean

In open ocean, the assessment of labile trace metals with passive samplers necessitates a different approach than in coastal areas because no moorings or buoys are present there. Hence, incubation experiments on board of the research vessel are the most convenient way for that goal. In the Northwest Pacific (VERTIGO cruise) and Southern Oceans (GEOTRACES cruise), large seawater samples (15 L) were collected with ultra clean techniques following GEOTRACES guidelines recommended for the International Polar Year (e.g. Bowie and Lohan [33]; Supplementary Information). Sampling locations in the Pacific and Southern oceans are shown on a map (Figure S1).

Table 2.

The non-available fractions are most pronounced for Fe, Cu and Mn, as can be seen from the percentages of the labile to total dissolved trace element concentrations at the different sites in the Pacific and Antarctic Oceans (Table 2). In the Northwest Pacific, total

dissolved (TD) and labile concentrations obtained by classic DGT are very similar for several elements (Co, Cd, Ni, Pb), while for others like Fe and Cu, the labile concentrations are by far lower than the TD concentrations (Table 3). Fe and Cu are known to be mainly present in the dissolved phase as colloids or as complexes that are strongly bound to ligands and, thus, unlikely to dissociate rapidly [34]. In the Southern Ocean, the TD and labile trace metal concentrations are mostly lower than in the Northwest Pacific. The labile concentrations of Co, Cd, Ni and Pb obtained by classic DGT (~10 nm) are substantially smaller than the total dissolved ones. Restricted (pore size of ~1 nm) DGTs were only applied in the Southern Ocean and for all trace metals, except Fe, concentrations can be calculated using specific diffusion coefficients for restricted pore size DGTs [20, 21]. For those concentration calculations, a ratio of diffusion coefficients in open pored to restricted pored DGTs of 1.3 to 1.4 (this ratio varies slightly in literature depending on the applied method) was used. Labile trace metal concentrations (Table 3) obtained with the open and restricted pored DGTs are very similar at stations 1-2, except for Mn but at stations 3-4, the restricted DGT concentrations of Mn, Co and Ni are much lower than the open pored DGTs. Further studies should clarify these differences. No other speciation studies using DGTs were carried out in open ocean. However, using electrochemical techniques, some speciation or complexation studies were performed in open ocean. Analyses on frozen samples were not taken into consideration, because freezing can change the composition of the sample. Organic complexation of dissolved Fe was measured by CLE-ACSV (Competitive Ligand Exchange-Adsorptive Cathodic Stripping Voltammetry) in North Atlantic, Southern and Central Pacific oceans [35–37]. The method allows to determine the concentration of the organic ligands and the conditional stability constant of the metal

complex. With this information and the TD concentration of Fe, the percentage of organic iron complexes in the dissolved phase can be calculated. Similar studies were carried out in the same oceanic areas for the other metals with ASV (Anodic Stripping Voltammetry) or with CLE-ACSV [38,39]. Stockdale et al. [40] used these studies on dissolved organic metal complexes in open ocean to calculate their average percentages (100*(organic-metal complex concentration/total dissolved metal concentration)) and obtained following results: 98.8% for Fe, 0.052% for Cd, 89.1% for Cu, 0.75% for Zn and 0.021% for Co. For the latter metal, Noble et al. [39] found, however, near complete complexation in surface waters due to a strong biological influence on cobalt cycling. The great advantage of DGT compared to the electrochemical methods is that with DGT in situ speciation is performed while the electrochemical methods require sample handling and are time consuming, increasing the risk of contamination and of change in sample composition.

Table 3.

Recently autonomous gliders (e.g. Slocum glider, Seaglider, SeaExplorer) came on the market with an operating depth range that varies between a few meters up to 1,000 m. They can perform sampling missions that last from weeks to months and cover thousands of kilometers but, the commercially available instruments are not designed for the determination of trace metals in the ocean. Therefore, our SeaExplorer (SEA010 from ALSEAMAR, France) was modified and equipped with DGTs [2], (Fig. 4). Metal concentrations in the different materials of the glider were very low and leaching at neutral pH is negligible (Tables S1 and S2). Moreover, all metal surfaces including the anodized Al plates were coated with a metal free resin from Reactive Resins Ltd, Cornwall, UK.

391 Auto-contamination was also verified by comparing the results obtained with DGT
392 samplers that are placed at the head and at the tail of the glider.

393 Figure 4.

394 In the Mediterranean Sea, two types of DGTs were applied (see cruise track on Figure
395 S2); one using a hydrogel diffusion layer of 0.8 mm (classic DGT measuring also partially
396 labile complexes) and the other using no hydrogel diffusion layer (fast DGT measuring
397 only full labile complexes). The DGTs mounted at the back of the glider did not accumulate
398 higher amounts of trace metals than those mounted at the front indicating that leaching
399 from the glider itself was not occurring (Table 4).

400 Table 4.

401 Labile trace metal concentrations obtained with the classic and the fast DGT samplers
402 are also compared (Table 4). The particularity of the technique implies that the fast DGT
403 accumulates a higher amount of metals on the resin in the same period of time than the
404 classic DGT, but the latter one also accumulates partially labile metal complexes which
405 have no time to dissociate in the thinner diffusive layer of the fast DGT. For Cu and Mn, a
406 difference is observed between the fast DGT results (only fully labile metal complexes are
407 taken up by the resin gel) and classic DGT results (also partially labile fractions that
408 dissociate in the hydrogel layer during sampling are bound by the resin gel). Using a multi-
409 method approach on samples from South San Francisco Bay, Donat et al. [41] showed that
410 different labile Cu fractions were present while this was not the case for Ni. Some trace
411 metals show a greater tendency to form a variety of labile complexes.

412 In literature, labile trace metal data in the Mediterranean Sea are not available,

however, labile Fe results (0.41 nmol L^{-1}) obtained with DGTs on the glider [2] are in the range 0.26-2.72 nmol L^{-1}, reported by Guieu et al. [42] for a similar area in the Mediterranean Sea and measured with FIA-CL. During several campaigns in the Southern Ocean also a good agreement was found between Fe-DGT results obtained via shipboard incubations in large sea water volumes with those obtained using FIA-CL [43].

4. CASE STUDY: BELGIAN COASTAL ZONE

4.1 Sampling, preparation, conditioning, storage and analyses of gel and resin

In a very turbid area at the entrance of Oostende harbor (51°12'55.84" N, 2°55'37.20" E), total dissolved and labile trace metal concentrations were assessed. For about 2 weeks, 6 DGT pistons were hold in a plastic cage 2 m below the water surface and the cage was held with a nylon rope which was anchored to the seabed using a weight and lifted to the surface with the help of a buoy. At the start of the experiment, 500 mL of filtered seawater (0.45 μm Durapore®, HVLP grade filter membrane) were filtered and stored in a Teflon bottle acidified with 1mL concentrated nitric acid for the determination of total dissolved trace metals.

All gel preparations were done under clean conditions within a Class-100 laminar flow cabinet. Preparations of diffusive gels (open pore and restricted pore diffusive gels), Chelex-100 resin gel, gel conditioning and DGT storage are routine procedures as described before [15,20].

The metal amounts captured by the Chelex resin gel are eluted with 1 mL of 1 mol

434 L^{-1} nitric acid and further 10 times diluted. The elution factor of the trace metals from
435 Chelex-100 was set equal to 0.8 and for Fe to 0.7 [15,16]. This solution was then analyzed
436 with High Resolution ICP-MS (Thermo Finnigan Element II) and quantified using
437 calibration curves of the trace metals made from appropriate dilutions of an acidified multi-
438 element stock solution (Merck, ICPMS standard XIII).

439 4.2 Total dissolved metal concentration (TD) determinations

440 Several methods exist for TD metal concentration determinations in seawater.
441 Preconcentration, which is often necessary for open ocean samples, can be performed with
442 a manual or an automated method. With the SeaFAST module (Elemental Scientific
443 Incorporated), that can be paired with an ICPMS in online mode, preconcentration of the
444 trace metal and removal of the salt matrix is performed in an automated way. The sample
445 is loaded on a column after adjustment of the pH and then eluted from the column by a
446 strong acid. The same steps can be carried out on the seawater sample in a manual way.
447 The extract that is eluted from the column is finally analyzed by ICPMS. In case the very
448 sensitive High Resolution ICPMS is used, a simple dilution method (for example a tenfold
449 dilution of the seawater sample) and direct analysis by HR-ICPMS can also be applied.
450 Calibration standards should always be matrix matched with the samples. In the Belgian
451 Coastal Zone we used the preconcentration method in a manual way.

452 4.3 Analytical characteristics of the DGTs

453 For each type of DGT and each trace metal, the blanks and LODs of the DGTs are
454 specific. Blanks are mainly determined by the purity of the strong acids, the filter
455 membrane, the polyacrylamide hydrogels and the resins. During field experiments in the

Belgian Coastal Zone (BCZ) and in the Mediterranean Sea, DGT sample to DGT blank signal ratios in the elution solution of the resin gels (elution concentrations in µg L^{-1}) are shown in Table 5. The blank DGTs have been submitted to the whole analytical procedure similar to the field samples except for the field deployment. In a similar way, a blank signal was assessed with DGTs containing ultra-pure nitric acid pre-cleaned Chelex resin instead of non-pretreated, commercially purchased resin. The latter results are included in Table 5 as cleaned blank.

Table 5.

All metals show higher ratios of field to blank elution solution concentrations in the BCZ than in the Mediterranean Sea except for Cd. In general, the ratios are higher than 8, except for Cd in the BCZ and Fe in the Mediterranean Sea. It is well-known that the labile (DGT) concentrations of Cd and Fe in seawater are low while on the other hand the blank values of the commercial resin in DGT can be high, resulting in such low ratios. However, when an acid pre-treatment of the resin is performed all blank concentrations decrease and the field to blank ratio of Fe in the Mediterranean Sea also exceeds a value of 10 [2]. During the same Mediterranean Sea cruise, the reproducibility of the DGT samplers was also investigated. For the classic (n=5) as well as the fast DGT (n=2) the relative standard deviation (RSD) for Cd, Mn, Co, Ni, Pb and Cu was below 20%. For Fe, that only could be determined with the fast DGT, the RSD amounts to 33%. During the SR3-GEOTRACES cruise in the Southern Ocean [3], RSD values for the restricted DGTs were assessed. At the stations with the highest concentrations (stations 1 and 2), RSD values varied between 8% for Mn and 24% for Pb and at the stations with the lowest

concentrations (stations 3 and 4) only the RSD value of Co was much higher, due to its very low restricted DGT concentration (4 pmol L^{-1}).

4.4 SPECIATION RESULTS IN BCZ

First the DBL was determined (an average DBL of 0.2 mm [44] was obtained), then labile metal concentrations were calculated. Results of total dissolved (TD) and labile metal (classic DGT) concentrations at station Oostende are shown in Fig. 5. For all elements, TD concentrations are higher than their labile concentrations: Pb, Cu and Cd show the lowest percentages of labile metal (< 20%), while Mn the highest (about 50%). For each of the considered trace elements, a certain fraction of their dissolved pool will thus likely be non-bioavailable to phytoplankton [34].

Figure 5.

5. INSIGHT INTO THE METAL LABILITY OF DIFFERENT POOLS

The special characteristics of the Oostende sampling site calls for a detailed analysis of the species that contribute to metal availability. Due to turbidity, metal released from suspended particles (via desorption or via dissolution) is expected to be very relevant in determining the metal availability and complement metal provided by complex dissociation. The problem of determining which species is contributing and the extent of such contribution is challenging due to the complexity of natural systems and the limited available information. However, the use of different DGT devices with

different thicknesses of the diffusion domain provides information that can be used to quantify labilities.

All metal complex species are classified into two pools: one, labelled inorganic ML_{in}, usually for smaller molecules (i.e. with larger diffusion coefficients) and another one, labelled organic ML_{org}. A third pool is that of the free metal ion. The diffusion coefficients of the pools, normalized with respect to the diffusion coefficient of free metal, are ε_{in} and ε_{org}. The total dissolved amount can then be split into three pools: the free metal pool c_M, an inorganic pool $c_{ML_{in}}$ and an organic pool $c_{ML_{org}}$.

$$c_{T,M} = c_M + c_{ML_{in}} + c_{ML_{org}} \quad (3)$$

Total dissolved metal concentrations ($c_{T,M}$) in the coastal North Sea area were from (unpublished results) and in the Mediterranean Sea from [45, 46]. Percentages of free metal, inorganic complexes (identified with pool in) and organic complexes (pool org) were taken from Stockdale et al. [40,47] (about open sea waters) for the Mediterranean Sea and from the measured medians in Table 2 of Stockdale et al. [48] (about coastal and estuarine waters) for the BCZ, given the expected similitude with the DGT-sampled zones. At equilibrium, due to an assumed excess of ligand, there are fixed proportionalities between the concentrations of each pool and the free metal concentration. For instance $K'_{in} = \frac{c_{ML_{in}}}{c_M}$

The kinetics of dissociation are assumed to follow a first order rate law with lumped dissociation rate constants $k_{d,in}$ and $k_{d,org}$

519 It has been shown [11] that, neglecting the impact of any size-fractionation due to
520 the pore size, the DGT concentration is a summation of the labile fractions, each
521 weighted by its normalized diffusion coefficient ε_j. For the fast (no hydrogel),
522 intermediate (0.4 mm thick diffusive hydrogel layer) and classic (0.8 mm thick diffusive
523 hydrogel layer) DGTs we can use respectively equations 4 to 6:

524 $$c_{\mathrm{DGT}}^{g0} = c_{\mathrm{M}}^{*} + \varepsilon_{\mathrm{in}}\xi_{\mathrm{in}}^{g0}c_{\mathrm{ML,in}}^{*} + \varepsilon_{\mathrm{org}}\xi_{\mathrm{org}}^{g0}\, c_{\mathrm{ML,org}}^{*} \quad (4)$$

525 $$c_{\mathrm{DGT}}^{g4} = c_{\mathrm{M}}^{*} + \varepsilon_{\mathrm{in}}\xi_{\mathrm{in}}^{g4}c_{\mathrm{ML,in}}^{*} + \varepsilon_{\mathrm{org}}\xi_{\mathrm{org}}^{g4}\, c_{\mathrm{ML,org}}^{*} \quad (5)$$

526 $$c_{\mathrm{DGT}}^{g8} = c_{\mathrm{M}}^{*} + \varepsilon_{\mathrm{in}}\xi_{\mathrm{in}}^{g8}c_{\mathrm{ML,in}}^{*} + \varepsilon_{\mathrm{org}}\xi_{\mathrm{org}}^{g8}\, c_{\mathrm{ML,org}}^{*} \quad (6)$$

527 Here ξ_j is the lability degree (between 0, for inert complexes, and 1, for fully
528 labile complexes) for each metal complex pool (j=in or org) in the classic, intermediate or
529 fast DGT (superscripts g8, g4 or g0). The lability degree quantifies the contribution of a
530 given complex (or ensemble of complexes) to the flux received by the DGT in
531 comparison with the maximum possible flux of this complex if it was fully labile.
532 Neglecting mixture effects [49], one can approximate the true ξ in a mixture of ligands
533 (or pools, in this case) with the ξ computed for the case where there is only one ligand
534 (i.e. one pool). Assuming excess of ligand, the lability degree depends on the dissociation
535 rate constant, the normalized diffusion coefficient of the complex and the thicknesses of
536 the diffusion (δ^{g}) and of the resin (δ^{r}=0.4 mm) domains as derived in [12],

$$\xi = 1 - \frac{\left(1+\varepsilon K'\right)}{\varepsilon K' + \frac{\delta^{g}}{\sqrt{\frac{D_{ML}}{k_d\left(1+\varepsilon K'\right)}}} \coth\left(\frac{\delta^{g}}{\sqrt{\frac{D_{ML}}{k_d\left(1+\varepsilon K'\right)}}}\right) + \frac{\delta^{g}}{\sqrt{\frac{D_{ML}}{k_d}}}\left(1+\varepsilon K'\right)\tanh\left(\frac{\delta^{r}}{\sqrt{\frac{D_{ML}}{k_d}}}\right)} \quad (7)$$

The aggregate thicknesses of the diffusion domain (δ^g) resulted from the summation of the filter thickness (0.125 mm), the DBL thickness (0.2 mm) and the diffusive gel thickness (0.8 mm for the classical DGT, 0.4 mm for the intermediate and 0 the fast ones).

Labilities and dissociation rates of the metal complex pools in the Belgian Coastal Zone were calculated for each pool and diffusion domain thickness, with equations 1 and 4 to 7, the percentages of free metal, inorganic complexes (identified with pool in) and organic complexes (pool org), and using the classic, intermediate and fast DGT results. The summation of the squares of the differences between the left and right-hand sides in equations (4)-(6) was minimized, with a weighting factor 0.1 for equation (4), 0.3 for equation (5) and a factor 0.6 for equation (6), given a larger impact of the DBL-thickness uncertainty when thinner diffusion gels are used. For BCZ, fixed diffusion coefficients have been taken: ε_{in}=1 (for all metals) and $D_{ML,org}$=3.48×10^{-6} cm^2 s^{-1} [50], before correcting for the temperature of 14.5ºC. Details for the Mediterranean Sea case can be found in [2].

Table 6.

All results for the Belgian Coastal Zone and the Mediterranean Sea are summarized in Table 6. As expected, labilities (for a given metal, sea and pool) increase when decreasing the thickness of the diffusive gel. For any metal and DGT type, the inorganic

pool is more labile than the organic one. The lability of the metal complexes seems in general in the Mediterranean Sea to be higher than in the Belgian Coastal Zone, which could suggest the presence of strong ligands coming from industrial activities in the harbor at the BCZ. Inorganic Cd, Ni, Co and Cu complexes in the Mediterranean Sea show a high lability in the classic DGT, with values around 90%. Inorganic Zn complexes in the BCZ also show a high lability in the classic DGT (99%), Cu decreases down to 68% and only half of the inorganic Pb complexes dissociate in the classic DGT. Organic Cu complexes have a similar lability in the BCZ as in the Mediterranean Sea. Finally, organic Fe complexes are more labile in the BCZ than in the Mediterranean Sea.

In this review paper it is shown that lability and dissociation kinetics of metal complex pools in the marine environment can be assessed with DGT samplers attached on an appropriate support and applying models based on the continuity equation in the diffusive domain of DGT. Such information is most valuable for (1) further understanding of trace metal bioavailability and toxicity effects in polluted coastal areas and (2) determination of trace nutrients limiting phytoplankton growth in HNLC oceanic areas.

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REFERENCES

[1] W. Sunda, Feedback Interactions between Trace Metal Nutrients and

Phytoplankton in the Ocean, Front. Microbiol. 3 (2012) 204. doi:10.3389/fmicb.2012.00204.

[2] W. Baeyens, Y. Gao, W. Davison, J. Galceran, M. Leermakers, J. Puy, P.-J. Superville, L. Beguery, In situ measurements of micronutrient dynamics in open seawater show that complex dissociation rates may limit diatom growth, Sci. Rep. 8 (2018) 16125. doi:10.1038/s41598-018-34465-w.

[3] W. Baeyens, A.R. Bowie, K. Buesseler, M. Elskens, Y. Gao, C. Lamborg, M. Leermakers, T. Remenyi, H. Zhang, Size-fractionated labile trace elements in the Northwest Pacific and Southern Oceans, Mar. Chem. 126 (2011) 108–113. doi:10.1016/J.MARCHEM.2011.04.004.

[4] A.R. Bowie, E.P. Achterberg, R.F.C. Mantoura, P.J. Worsfold, Determination of sub-nanomolar levels of iron in seawater using flow injection with chemiluminescence detection, Anal. Chim. Acta. 361 (1998) 189–200. doi:10.1016/S0003-2670(98)00015-4.

[5] W. Baeyens, G. Gillain, G. Decadt, I. Elskens, Trace metals in coastal waters of the eastern North Sea. I. Analyses and short term distributions, Oceanol. Acta. 10 (1987) 169–179.

[6] C. Zhou, S. van de Velde, W. Baeyens, Y. Gao, Comparison of Chelex based resins in diffusive gradients in thin-film for high resolution assessment of metals, Talanta. 186 (2018) 397–405. doi:10.1016/j.talanta.2018.04.085.

[7] B. Docekal, M. Gregusova, Segmented sediment probe for diffusive gradient in thin films technique, Analyst. 137 (2012) 502–507. doi:10.1039/C1AN15701A.

[8] A.A. Menegário, L.N.M. Yabuki, K.S. Luko, P.N. Williams, D.M. Blackburn, Use of diffusive gradient in thin films for in situ measurements: A review on the progress in chemical fractionation, speciation and bioavailability of metals in waters, Anal. Chim. Acta. 983 (2017) 54–66. doi:10.1016/J.ACA.2017.06.041.

[9] Y. Gao, W. Baeyens, S. De Galan, A. Poffijn, M. Leermakers, Mobility of radium and trace metals in sediments of the Winterbeek: Application of sequential extraction and DGT techniques, Environ. Pollut. 158 (2010) 2439–2445. doi:10.1016/J.ENVPOL.2010.03.022.

[10] J. Drozdzak, M. Leermakers, Y. Gao, V. Phrommavanh, M. Descostes, Evaluation and application of Diffusive Gradients in Thin Films (DGT) technique using Chelex®-100, Metsorb™ and Diphonix® binding phases in uranium mining environments, Anal. Chim. Acta. 889 (2015) 71–81. doi:https://doi.org/10.1016/j.aca.2015.07.057.

[11] J. Galceran, J. Puy, Interpretation of diffusion gradients in thin films (DGT) measurements: a systematic approach, Environ. Chem. 12 (2015) 112–122. https://doi.org/10.1071/EN14068.

[12] S. Mongin, R. Uribe, J. Puy, J. Cecília, J. Galceran, H. Zhang, W. Davison, Key Role of the Resin Layer Thickness in the Lability of Complexes Measured by

DGT, Environ. Sci. Technol. 45 (2011) 4869–4875. doi:10.1021/es200609v.

[13] R. Uribe, S. Mongin, J. Puy, J. Cecília, J. Galceran, H. Zhang, W. Davison, Contribution of Partially Labile Complexes to the DGT Metal Flux, Environ. Sci. Technol. 45 (2011) 5317–5322. doi:10.1021/es200610n.

[14] H. Zhang, W. Davison, Diffusional characteristics of hydrogels used in DGT and DET techniques, Anal. Chim. Acta. 398 (1999) 329–340. doi:https://doi.org/10.1016/S0003-2670(99)00458-4.

[15] H. Zhang, W. Davison, Performance Characteristics of Diffusion Gradients in Thin Films for the in Situ Measurement of Trace Metals in Aqueous Solution, Anal. Chem. 67 (1995) 3391–3400. doi:10.1021/ac00115a005.

[16] Y. Gao, M. Leermakers, C. Gabelle, P. Divis, G. Billon, B. Ouddane, J.-C. Fischer, M. Wartel, W. Baeyens, High-resolution profiles of trace metals in the pore waters of riverine sediment assessed by DET and DGT, Sci. Total Environ. 362 (2006) 266–277. doi:10.1016/J.SCITOTENV.2005.11.023.

[17] W. Davison, H. Zhang, Principles of measurements in simple solutions, in: W. Davison (Ed.), Diffus. Gradients Thin-Films Environ. Meas., Cambridge university press, 2016: pp. 10–31.

[18] A. Altier, M. Jiménez-Piedrahita, C. Rey-Castro, J. Cecilia, J. Galceran, J. Puy, Accumulation of Mg to Diffusive Gradients in Thin Films (DGT) Devices: Kinetic and Thermodynamic Effects of the Ionic Strength, Anal. Chem. 88 (2016) 10245–10251. doi:10.1021/acs.analchem.6b02961.

[19] H.P. van Leeuwen, Revisited: DGT speciation analysis of metal–humic acid complexes, Environ. Chem. 13 (2016) 84–88. https://doi.org/10.1071/EN15066.

[20] S. Scally, W. Davison, H. Zhang, Diffusion coefficients of metals and metal complexes in hydrogels used in diffusive gradients in thin films, Anal. Chim. Acta. 558 (2006) 222–229. doi:10.1016/J.ACA.2005.11.020.

[21] A.H. Shiva, W.W. Bennett, D.T. Welsh, P.R. Teasdale, In situ evaluation of DGT techniques for measurement of trace metals in estuarine waters: a comparison of four binding layers with open and restricted diffusive layers, Environ. Sci. Process. Impacts. 18 (2016) 51–63. doi:10.1039/C5EM00550G.

[22] H.P. van Leeuwen, R.M. Town, J. Buffle, R.F.M.J. Cleven, W. Davison, J. Puy, W.H. van Riemsdijk, L. Sigg, Dynamic Speciation Analysis and Bioavailability of Metals in Aquatic Systems, Environ. Sci. Technol. 39 (2005) 8545–8556. doi:10.1021/es050404x.

[23] C.S. Chapman, C.M.G. van den Berg, Anodic Stripping Voltammetry Using a Vibrating Electrode, Electroanalysis. 19 (2007) 1347–1355. doi:10.1002/elan.200703873.

[24] J. Puy, R. Uribe, S. Mongin, J. Galceran, J. Cecília, J. Levy, H. Zhang, W. Davison, Lability Criteria in Diffusive Gradients in Thin Films, J. Phys. Chem. A. 116 (2012) 6564–6573. doi:10.1021/jp212629z.

[25] M.S. Kim, M.S. Choi, C.-K. Kim, Monitoring trace metals in seawater using a diffusive gradient in thin film probe in Ulsan Bay, East Sea, Korea: Comparison with transplanted mussels, Ocean Sci. J. 51 (2016) 169–182. doi:10.1007/s12601-016-0015-y.

[26] M. Vannuci-Silva, J.M. de Souza, F.F. de Oliveira, M.A.G. de Araújo, E. Francioni, C.E. Eismann, C.H. Kiang, J.S. Govone, M.J. Belzunce-Segarra, A.A. Menegário, Bioavailability of Metals at a Southeastern Brazilian Coastal Area of High Environmental Concern Under Anthropic Influence: Evaluation Using Transplanted Bivalves (Nodipecten nodosus) and the DGT Technique, Water, Air, Soil Pollut. 228 (2017) 222. doi:10.1007/s11270-017-3387-4.

[27] J. Søndergaard, L. Bach, K. Gustavson, Measuring bioavailable metals using diffusive gradients in thin films (DGT) and transplanted seaweed (Fucus vesiculosus), blue mussels (Mytilus edulis) and sea snails (Littorina saxatilis) suspended from monitoring buoys near a former lead–zinc mine in We, Mar. Pollut. Bull. 78 (2014) 102–109. doi:10.1016/J.MARPOLBUL.2013.10.054.

[28] M. Schintu, B. Marras, L. Durante, P. Meloni, A. Contu, Macroalgae and DGT as indicators of available trace metals in marine coastal waters near a lead–zinc smelter, Environ. Monit. Assess. 167 (2010) 653–661. doi:10.1007/s10661-009-1081-8.

[29] A. Amirbahman, D.I. Massey, G. Lotufo, N. Steenhaut, L.E. Brown, J.M. Biedenbach, V.S. Magar, Assessment of mercury bioavailability to benthic macroinvertebrates using diffusive gradients in thin films (DGT), Environ. Sci. Process. Impacts. 15 (2013) 2104–2114. doi:10.1039/C3EM00355H.

[30] R.J.K. Dunn, P.R. Teasdale, J. Warnken, R.R. Schleich, Evaluation of the Diffusive Gradient in a Thin Film Technique for Monitoring Trace Metal Concentrations in Estuarine Waters, Environ. Sci. Technol. 37 (2003) 2794–2800. doi:10.1021/es026425y.

[31] A.-M. Cindrić, N. Cukrov, G. Durrieu, C. Garnier, I. Pižeta, D. Omanović, Evaluation of Discrete and Passive Sampling (Diffusive Gradients in Thin-films – DGT) Approach for the Assessment of Trace Metal Dynamics in Marine Waters – a Case Study in a Small Harbor, Croat. Chem. Acta. 90 (2017) 177–185. doi:10.5562/cca3163.

[32] J.E. Sherwood, D. Barnett, K. Dover, J. Howitt, H. Ii, P. Kew, J. Mondon, Deployment of DGT units in marine waters to assess the environmental risk from a deep sea tailings outfall, Anal. Chim. Acta. 652 (2009) 215–223. doi:10.1016/J.ACA.2009.06.009.

[33] A.R. Bowie, M.C. Lohan, Practical Guidelines for the Analysis of Seawater, in: O. Wurl (Ed.), Determ. Iron Seawater, Taylor & Francis Group, 2009: pp. 235–257.

[34] F.M.M. Morel, N.M. Price, The Biogeochemical Cycles of Trace Metals in the Oceans, Science (80-.). 300 (2003) 944–947. doi:10.1126/science.1083545.

[35] E.L. Rue, K.W. Bruland, Complexation of iron(III) by natural organic ligands in

the Central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method, Mar. Chem. 50 (1995) 117–138. doi:10.1016/0304-4203(95)00031-L.

[36] L.J.A. Gerringa, S. Blain, P. Laan, G. Sarthou, M.J.W. Veldhuis, C.P.D. Brussaard, E. Viollier, K.R. Timmermans, Fe-binding dissolved organic ligands near the Kerguelen Archipelago in the Southern Ocean (Indian sector), Deep Sea Res. Part II Top. Stud. Oceanogr. 55 (2008) 606–621. doi:10.1016/J.DSR2.2007.12.007.

[37] K.N. Buck, B. Sohst, P.N. Sedwick, The organic complexation of dissolved iron along the U.S. GEOTRACES (GA03) North Atlantic Section, Deep Sea Res. Part II Top. Stud. Oceanogr. 116 (2015) 152–165. doi:10.1016/J.DSR2.2014.11.016.

[38] K.W. Bruland, Complexation of cadmium by natural organic ligands in the central North Pacific, Limnol. Oceanogr. 37 (1992) 1008–1017. doi:10.4319/lo.1992.37.5.1008.

[39] A.E. Noble, D.C. Ohnemus, N.J. Hawco, P.J. Lam, M.A. Saito, Coastal sources, sinks and strong organic complexation of dissolved cobalt within the US North Atlantic GEOTRACES transect GA03, Biogeosciences. 14 (2017) 2715–2739. doi:10.5194/bg-14-2715-2017.

[40] A. Stockdale, E. Tipping, S. Lofts, R.J.G. Mortimer, Effect of Ocean Acidification on Organic and Inorganic Speciation of Trace Metals, Environ. Sci. Technol. 50 (2016) 1906–1913. doi:10.1021/acs.est.5b05624.

[41] J.R. Donat, K.A. Lao, K.W. Bruland, Speciation of dissolved copper and nickel in South San Francisco Bay: a multi-method approach, Anal. Chim. Acta. 284 (1994) 547–571. doi:10.1016/0003-2670(94)85061-5.

[42] C. Guieu, Y. Bozec, S. Blain, C. Ridame, G. Sarthou, N. Leblond, Impact of high Saharan dust inputs on dissolved iron concentrations in the Mediterranean Sea, Geophys. Res. Lett. 29 (2002) 14–17. doi:10.1029/2001GL014454.

[43] E.C.V. Butler, J.E. O'Sullivan, R.J. Watson, A.R. Bowie, T.A. Remenyi, D. Lannuzel, Trace metals Cd, Co, Cu, Ni, and Zn in waters of the subantarctic and Polar Frontal Zones south of Tasmania during the 'SAZ-Sense' project, Mar. Chem. 148 (2013) 63–76. doi:10.1016/J.MARCHEM.2012.10.005.

[44] W. Guo, K. Van Langenhove, T. Vandermarken, M. Denison, M. Elskens, W. Baeyens, Y. Gao, In situ measurement of estrogenic activity in various aquatic systems using diffusive gradients in thin-film coupled with ERE-CALUX bioassay, Environ. Int. In press (n.d.).

[45] A.M. Ebling, W.M. Landing, Sampling and analysis of the sea surface microlayer for dissolved and particulate trace elements, Mar. Chem. 177 (2015) 134–142. doi:10.1016/J.MARCHEM.2015.03.012.

[46] G. Dulaquais, H. Planquette, S. L'Helguen, M.J.A. Rijkenberg, M. Boye, The biogeochemistry of cobalt in the Mediterranean Sea, Global Biogeochem. Cycles.

31 (2017) 377–399. doi:10.1002/2016GB005478.

[47] A. Stockdale, E. Tipping, J. Hamilton-Taylor, S. Lofts, Trace metals in the open oceans: speciation modelling based on humic-type ligands, Environ. Chem. 8 (2011) 304–319. https://doi.org/10.1071/EN11004.

[48] A. Stockdale, E. Tipping, S. Lofts, Dissolved trace metal speciation in estuarine and coastal waters: Comparison of WHAM/Model VII predictions with analytical results, Environ. Toxicol. Chem. 34 (2015) 53–63. doi:10.1002/etc.2789.

[49] A. Altier, M. Jiménez-Piedrahita, R. Uribe, C. Rey-Castro, J. Cecília, J. Galceran, J. Puy, Effects of a mixture of ligands on metal accumulation in diffusive gradients in thin films (DGT), Environ. Chem. 15 (2018) 183–193. https://doi.org/10.1071/EN17232.

[50] J. Balch, C. Guéguen, Effects of molecular weight on the diffusion coefficient of aquatic dissolved organic matter and humic substances, Chemosphere. 119 (2015) 498–503. doi:10.1016/J.CHEMOSPHERE.2014.07.013.

Supplementary Information

Figures: Fig. S1 and Fig. S2

Tables: Table T1 and Table T2

Figure Legend

Figure 1: Total dissolved metal pool: relation between free metal, labile and non-labile metal complex pools

Figure 2: Schematic representation of a classic DGT assembly (in case of the reversed DGT, the diffusive gel is omitted)

Figure 3: Functioning of the DGT: A) Metal complexes are labile. B) Metal complexes are poorly labile. C) Metals are adsorbed on colloids

Figure 4: View of the modified SeaExplorer glider: the white circles in the nose are the DGTs

768 Figure 5: Total dissolved (SPE extracted samples) and labile (classic DGT) trace metal
769 concentrations at station Oostende in the Belgian Coastal Zone

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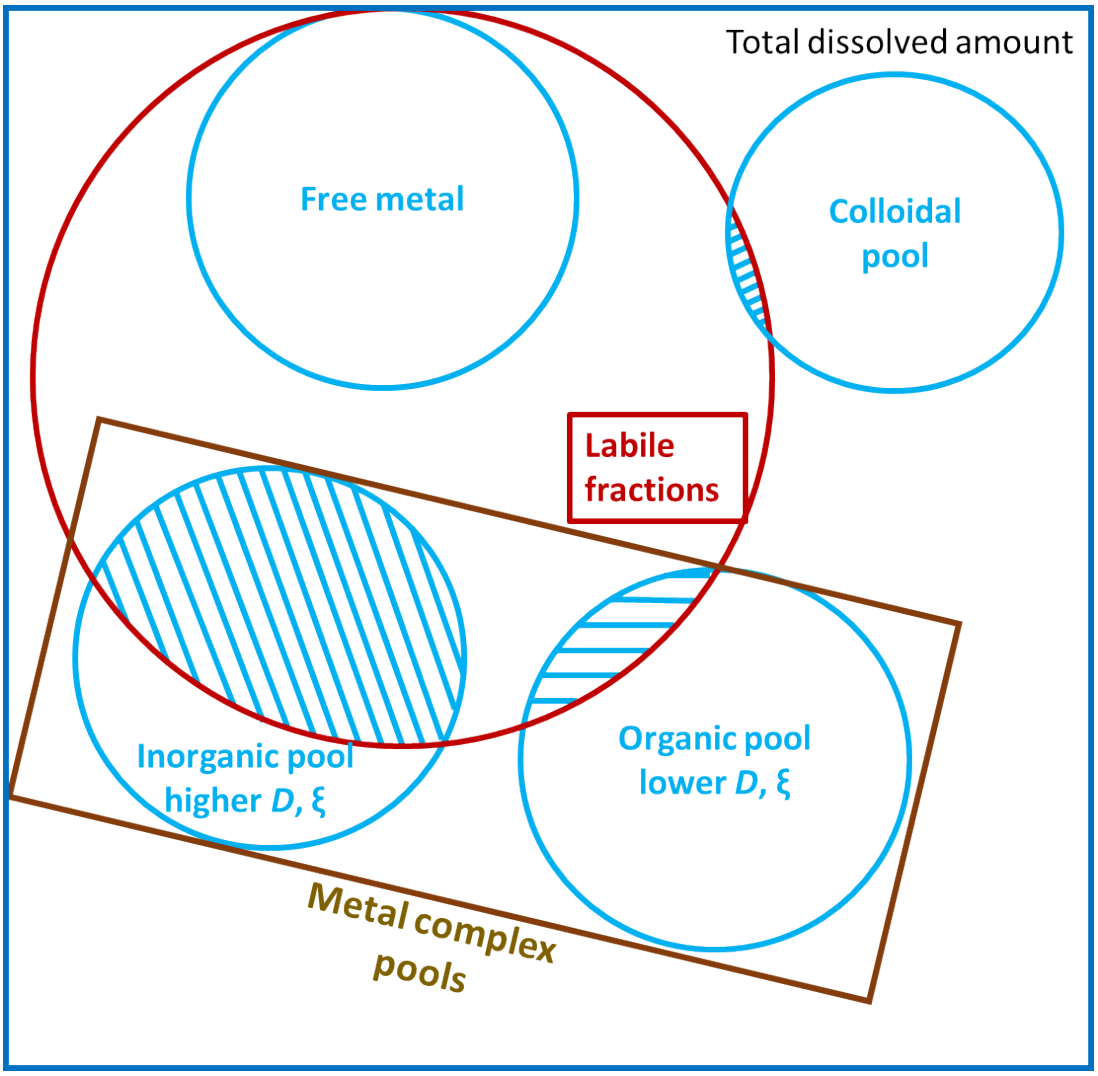

Figure 1: Total dissolved metal pool: relation between free metal, labile and non-labile metal complex pools

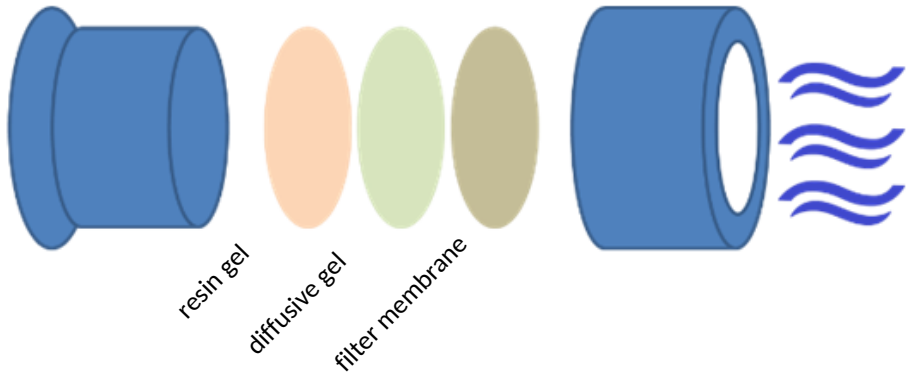

Figure 2: Schematic representation of a classic DGT assembly (in case of the reversed DGT, the diffusive gel is omitted)

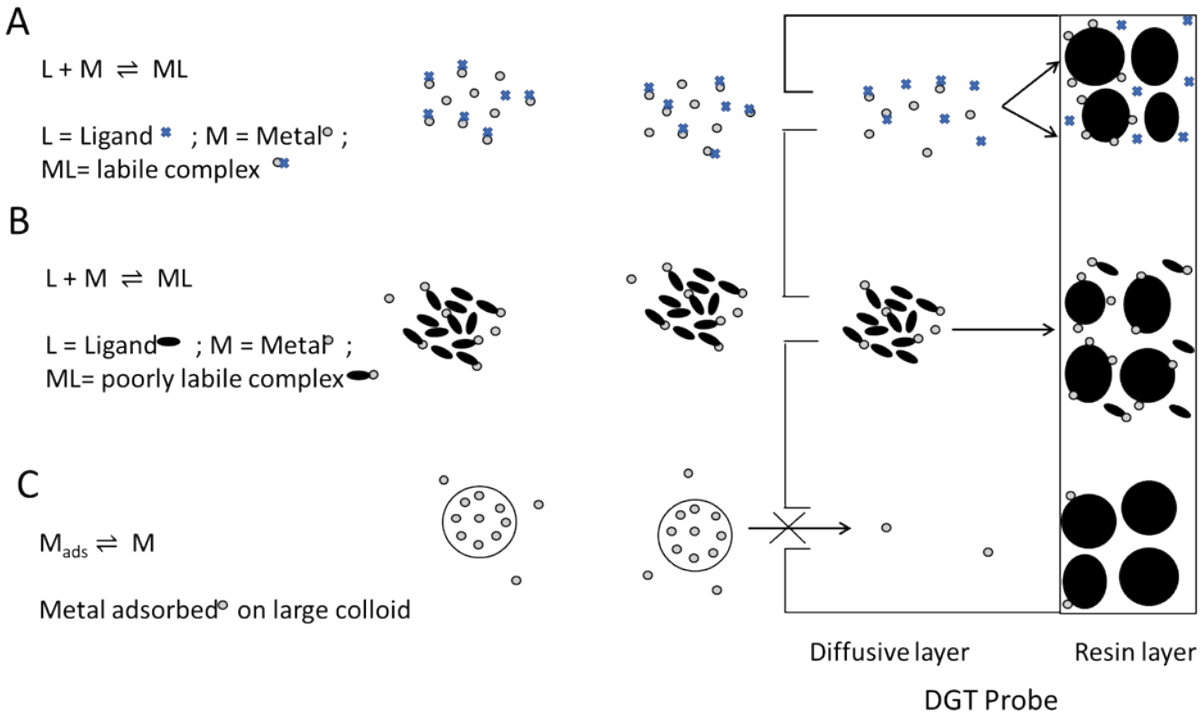

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Figure 4: View of the modified SeaExplorer glider

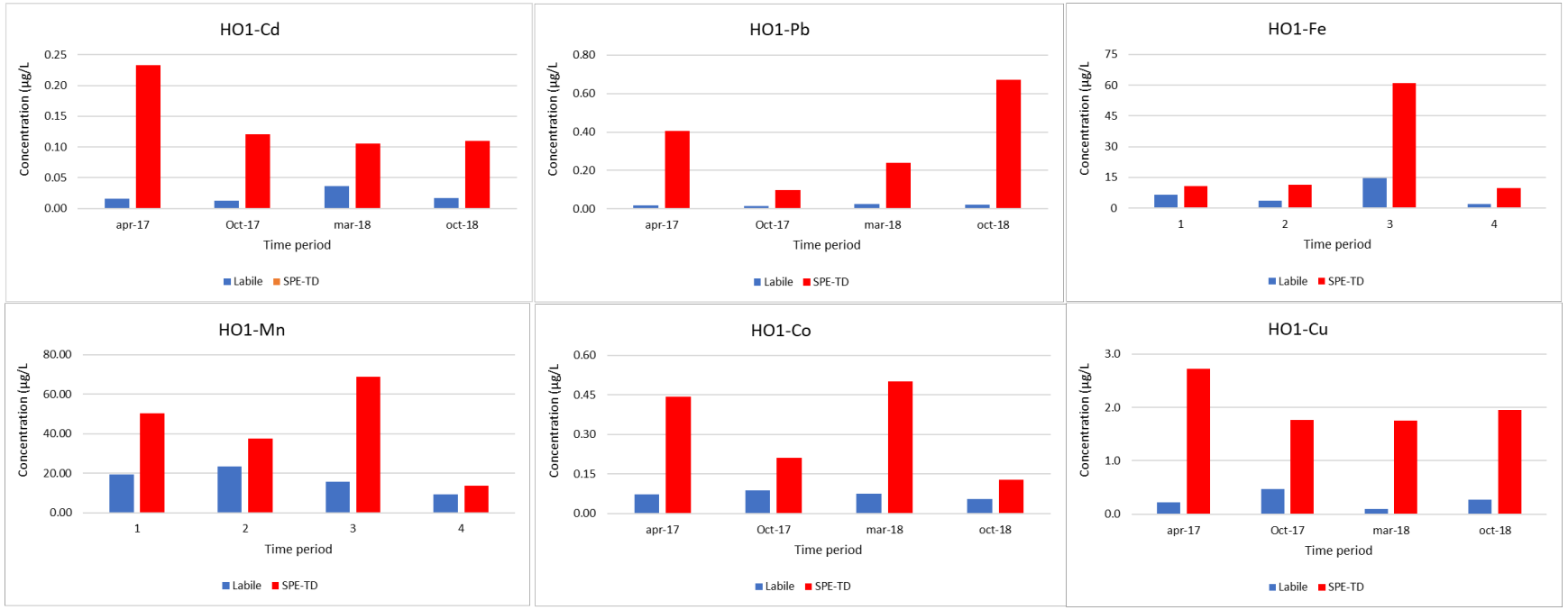

Figure 5: Total dissolved (SPE extracted samples) and labile (classic DGT) trace metal concentrations at station Oostende in the Belgian Coastal Zone

Table 1: Characteristics of the different DGT sampler types.

	Classic DGT	Fast DGT	Restricted pore DGT
Thickness filter	0.125 mm	0.125 mm	0.125 mm
Thickness Hydrogel layer	0.8 mm	-	0.8 mm
Thickness Resin gel layer	0.4 mm	0.4 mm	0.4 mm
Pore size Hydrogel layer	~10 nm	-	~1 nm

Table 2: Percentages of labile (classic DGT) to total dissolved (TD) trace element concentrations

VERTIGO station K2

	Fe	Cu	Co	Cd	Mn	Ni	Pb
% Labile/TD	10	21	100	100	44	100	82

ANTAR SR3-GEOTRACES, stations # 1 & 2

	Fe	Cu	Co	Cd	Mn	Ni	Pb
% Labile/TD	27	35	47	61		48	45

ANTAR SR3-GEOTRACES, stations # 3 & 4

	Fe	Cu	Co	Cd	Mn	Ni	Pb
% Labile/TD	7	14	33	24		40	16

Table 3: Total dissolvable (TD) and labile element concentrations (classic) in the Northwest Pacific (VERTIGO) and Southern Oceans (SR3-GEOTRACES). TD = Total Dissolved, ~10 nm respectively ~1 nm = labile fraction obtained with the classic DGT for a pore size of about 10 nm respectively 1 nm

Element concentrations in nmol L^{-1} (VERTIGO)

	P	Fe	Cu	Co	Cd	Mn	Ni	Pb
TD average	2000	3.1	1.5	0.050	0.41	1.69	4.02	0.056
STD		± 0.5	±0.3	±0.008	±0.05	±0.23	±0.76	±0.012
~ 10 nm average		0.30	0.31	0.056	0.40	0.74	4.04	0.046
STD		±0.08	±0.03	±0.005	±0.03	±0.08	±0.32	±0.005

Element concentrations in nmol L^{-1} (SR3-GEOTRACES, stations # 1 & 2)

	P	Fe	Cu	Co	Cd	Mn	Ni	Pb
TD average	500-1700	2.5	1.03	0.0285	0.24		4.7	0.088
STD		±0.4	±0.15	±0.0045	±0.04		±0.7	±0.012
~ 10 nm average		0.68	0.36	0.0135	0.147	0.28	2.30	0.040
STD		±0.09	±0.05	±0.0020	±0.023	±0.05	±0.35	±0.009
~ 1 nm average			0.42	0.0160	0.143	0.11	2.7	0.043
STD			±0.06	±0.0027	±0.028	±0.01	±0.4	±0.011

Element concentrations in nmol L^{-1} (SR3-GEOTRACES, stations # 3 & 4)

	P	Fe	Cu	Co	Cd	Mn	Ni	Pb
TD average	500-1700	2.4	0.80	0.0255	0.112		2.91	0.085
STD		±0.4	±0.08	±0.0040	±0.015		±0.29	±0.014
~ 10 nm average		0.165	0.11	0.0085	0.0265	0.23	1.17	0.014
STD		±0.025	±0.02	±0.0010	±0.0040	±0.04	±0.17	±0.002

~ 1 nm average	0.12	0.0026	0.0228	0.098	0.57	0.010
STD	±0.02	±0.0020	±0.0042	±0.014	±0.08	±0.002

Table 4: cDGT results obtained with classic (1.125 mm diffusive domain) and fast (0.325 mm diffusive domain) DGTs and at the head and the tail of the glider. Concentrations in nmol L^{-1}

	Cd	Mn	Co	Ni	Cu	Fe
Head DGT-fast	0.049	0.97	0.058	2.96	0.41	0.47
RSD	6%	17%	8%	9%	9%	33%
Head DGT-classic	0.062	1.69	0.064	3.2	0.94	
RSD	14%	19%	12%	13%	19%	
Tail DGT-classic	0.061	1.25	0.062	3.1	0.73	
RSD	18%	13%	13%	10%	2%	

Table 5: Sample to blank ratios in the elution solution of the resin gel (fast DGT). BCZ is Belgian Coastal Zone and MS is Mediterranean Sea

	Mn	Co	Ni	Cu	Zn	Cd	Fe	Pb
Sample/blank ratio BCZ	970	305	98	13	15	3	8	26
Sample/blank ratio MS	38	38	36	9	8	14	4	26
Sample/cleaned blank ratio MS	93	120	380	28	21	130	12	95

Table 6: Calculated lability degrees (ξ) and dissociation rates (k_d) of metal complexes in the Mediterranean Sea and the Belgian coastal Zone (BCZ). $c_{T,M}$ is the total dissolved metal concentration (Co from Dulaquais et al. (2017) and the other metals from Ebling and Landing (2015) for the Mediterranean Sea and our data for the BCZ. Percentages of each pool (free, inorganic and organic) are from Stockdale et al. (2011; 2015; 2016). in stands for inorganic and org for organic. Superscript *g*0 indicates no hydrogel layer, g4 0.4 mm and g8 0.8 mm hydrogel layer

Element	$c_{T,M}$	Free metal	DGT-fast (g0)	DGT-interm (g4)	DGT-classic (g8)	ξ_{in}^{g0}	ξ_{org}^{g0}	ξ_{in}^{g4}	ξ_{org}^{g4}	ξ_{in}^{g8}	ξ_{org}^{g8}	$k_{d,in}$	$k_{d,org}$
	nmol L^{-1}	nmol L^{-1}	nmol L^{-1}	nmol L^{-1}	nmol L^{-1}							s^{-1}	s^{-1}
Med. Sea													
Cd	0.066	0.0029	0.049		0.062	0.694	-			0.89	-	0.019	-
Fe	5.54	5.3×10^{-13}	0.47		-	-	0.152			-	-	-	0.00043
Ni	4.9	0.49	2.95		3.18	0.88	-			0.96	-	0.089	-
Co	0.12	0.019	0.058		0.064	0.81	-			0.94	-	0.019	-
Cu	4.53	0.0044	0.41		0.98	0.70	0.14			0.89	0.36	0.018	0.00035
BCZ													
Pb	0.473	0.0047	0.0458	0.0644	0.073	0.22	3.8×10^{-10}	0.40	1.1×10^{-9}	0.51	2.0×10^{-9}	0.0012	5.9×10^{-13}
Fe	205	$1.6\text{x}10^{-9}$	37.3	66.6	72.9	-	0.34	-	0.53	-	0.64	-	0.00124
Ni	39.5	5.53	5.65	8.17	9.91	0.04	0.04	0.11	0.10	0.18	0.17	0.00011	6.4×10^{-5}
Cu	27.7	0.070	3.62	7.61	8.09	0.38	0.14	0.58	0.27	0.68	0.37	0.00242	0.000297
Zn	180	10.8	33.6	45.0	50.2	0.96	0.07	0.98	0.16	0.99	0.25	1.42	0.00013

SUPPLEMENTARY INFORMATION:

Labile trace metal concentrations in marine environments: from coastal to open ocean areas.

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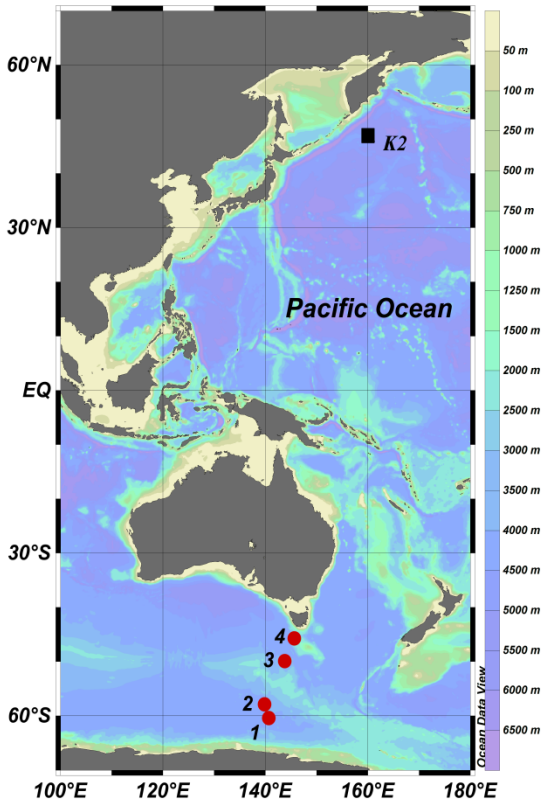

Figure S1: sampling locations in the Pacific (K2) and the Southern oceans (1 to 4).

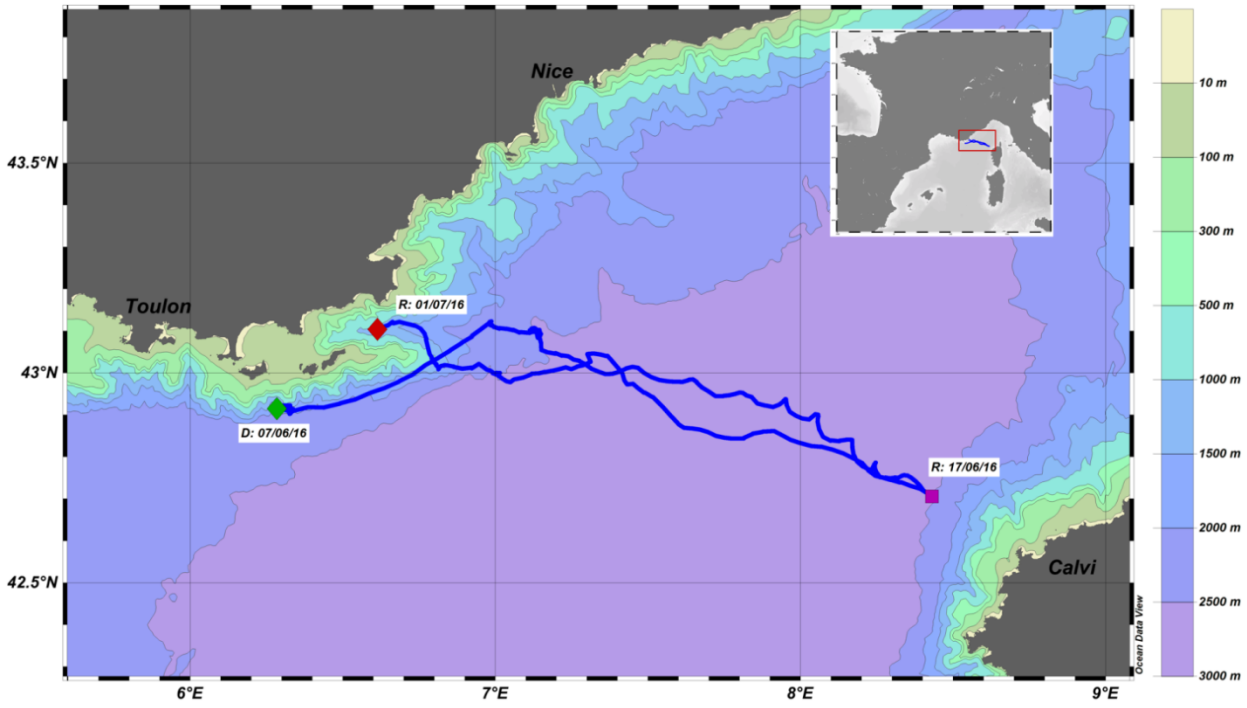

Figure S2: Cruise track of the SeaExplorer glider from Isle du Levant to Corsica and back

Table S1: Composition of Al and anodized Al plates.

	Al	Si	S	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
	%	*%*	*%*	*mg/g*	*mg/g*	*µg/g*	*µg/g*	*mg/g*	*mg/g*	*µg/g*	*µg/g*
anodized Al plate	75	4.5	18.6	0.31	2.3	1.8	33	1.6	0.36	0.11	7.5
Al plate	99	0.01	0.02	0.29	1.9	1.4	24	1.5	0.20	0.08	5.2

Table S2: Trace metals in polyurethane (PU) material and in leaching solutions.

		Cd	Pb	Al	Mn	Fe	Co	Ni	Cu	Zn
PU-total	$\mu g/g$	0.0038	0.022	9.1	4.0	114	0.026	0.39	0.39	38
HCl 0.1 N	$\mu g/g$	0.0009	0.022	0.6	0.01	0.8	0.001	0.03	0.08	1.3
HAc 1 M	$\mu g/g$	0.0001	0.009	0.09	0.01	0.3	0.0004	0.01	0.03	1.0
HNO3 1 M	$\mu g/g$	0.0006	0.021	0.6	0.02	0.7	0.002	0.04	0.10	0.6

Sampling information: In the Northwest Pacific Ocean (VERTIGO cruise), pre-cleaned Teflon-lined Niskin bottles (10 L) deployed off a Kevlar cable were used to collect surface sea water, up to a depth of 50 m. Additional surface samples were collected from an inflatable raft upstream from the ship, using clean sampling procedures. Fifteen liters of seawater were directly poured into a pre-cleaned high-density polymer vessel (20 L) containing 5 DGT devices (classic DGTs). The 20 L container was stored between 5 and 10ºC and the seawater was frequently homogenized by gentle shaking the container. After 12 days, the seawater in the container was refreshed with a new 15 L seawater sample. After 22 days, the DGT probes were then recovered, stored in sealed double layered plastic bags and kept at 4ºC, together with 2 blank DGT probes. At the start of the incubation, after 12 days and at the end of the experiment, two 0.5 L aliquots (unfiltered seawater) were taken from the container and stored in Teflon bottles (pH~3) for later analysis of TD trace metal concentrations.

In the Southern Ocean (SR3-GEOTRACES cruise), Teflon-lined Niskin-1010X bottles (5 L) were deployed on an autonomous 1018 intelligent rosette system specially adapted for trace metal work (General Oceanics, USA) and suspended on Kevlar rope. Two size fractions of labile trace metals were sampled: size fraction ~10 nm (open-pored DGT) and size fraction ~1 nm (restricted-pored DGT). The DGT experiments were run in the same way as during the VERTIGO-K2 cruise, except that they lasted for 7 weeks (i.e., DGTs remained in the sample solution for 7 weeks) and at a

temperature of 4 °C. At the end of each DGT experiment, two 0.5 L aliquots (unfiltered seawater) were also taken from the container and stored in Teflon bottles (pH~3) for later TD analysis. The DGT probes were recovered, stored in double zipped plastic bags and kept at 4°C, together with 2 blank DGT probes.